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Some Characteristics of the Action of Dapsone on Multiplication of *Mycobacterium leprae* in the Mouse*

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In a number of experiments, male BALB/c mice were inoculated with *Mycobacterium leprae* and administered dapsone (4,4′-diaminodiphenylsulphone, DDS) incorporated into the mouse chow in concentrations of 10^{-2} to $10^{-4.5}$ g% for periods of about 90 days during logarithmic multiplication of the organisms. Both the duration of the delay between beginning treatment and the onset of inhibition of bacterial multiplication and the duration of the delay between cessation of treatment and resumption of bacterial multiplication were dependent on the dosage of DDS. The number of doublings of *M. leprae* after the start of DDS treatment appeared more sensitive to minor variations of DDS concentration than the duration of the delay of resumption of multiplication after treatment was stopped.

Introduction

The activity of dapsone (4,4′-diaminodiphenylsulphone, DDS) against *Mycobacterium leprae* has been intensively studied in mice. When DDS, incorporated into the mouse chow, is administered for periods of 60-90 days during logarithmic multiplication of the organisms in the mouse foot-pad [Shepard's "kinetic" method (Shepard, 1967, 1969)], the antimicrobial activity of the drug may be characterized according to 2 criteria:

(1) the duration of the delay between beginning treatment of the mice with an effective dose of DDS and the onset of inhibition of bacterial multiplication; and
(2) the duration of the delay between cessation of treatment and the resumption of bacterial multiplication.

The delay between beginning treatment and the onset of inhibition is shorter, and

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that between cessation of treatment and the resumption of multiplication is longer when DDS has been administered in a larger dosage (Shepard, 1967, 1969).

During the past 8 years, we have conducted a number of experiments in which DDS was administered to *M. leprae*-infected mice. Although we observed the same characteristics of antimicrobial activity of DDS noted by Shepard (1967, 1969), it was also apparent that the quality of inhibition of multiplication of *M. leprae* varied from experiment to experiment in which the dosage of DDS, the strain of *M. leprae,* and the strain of inbred mice were not varied. We have analysed the results of these experiments in an attempt to explain the variation of drug effect. An important cause of the variation of drug effect appears to be minor variation of the dosage of DDS revealed by measurements of the concentration of DDS in the plasma of mice sacrificed for harvests of *M. leprae.*

Materials and Methods

DDS, purchased from K & K Laboratories, Inc., Hollywood, California, U.S.A., was incorporated into the mouse chow (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Illinois, U.S.A.) by means of a liquid-solid twin-shell blender (Patterson-Kelly Co., East Stroudsburg, Pennsylvania, U.S.A.). The strain of *M. leprae* used in all experiments was a "fast" strain (Shepard and McRae, 1971) that had been isolated by C. C. Shepard, Center for Disease Control, Atlanta, Georgia, U.S.A., from an untreated patient with lepromatous leprosy, and was subsequently carried through many mouse passages both in Shepard's laboratory and in ours. Locally-bred weanling male BALB/c mice were employed in all of the experiments.

Mice were inoculated and *M. leprae* were harvested and enumerated by established methods (Shepard, 1960; Shepard and McRae, 1968). Administration of DDS in concentrations of $10^{-4.5}$ to 10^{-2} g% was carried out for periods of about 90 days, beginning usually about 60 days after inoculation; in 4 experiments, DDS administration was begun about 75 days after inoculation. Harvests of *M. leprae* were carried out from the pooled tissues of 4-8 foot-pads of untreated mice at intervals beginning about 100 days after inoculation, in order to define the logarithmic portion of the growth curve. Similar harvests were carried out from the foot-pad tissues of DDS-treated mice at least once late during the period of treatment, and at intervals thereafter until the *M. leprae* had multiplied to the level of 10^6 organisms per foot-pad, or until no mice remained. The regression of the $\log_{10}$ number of acid-fast bacilli (AFB) per foot-pad on the time in days after inoculation was calculated (Goldstein, 1964), assuming a constant doubling time during logarithmic multiplication of 12 days (Levy, 1976), to represent the phase of logarithmic growth of *M. leprae.* When mice were to be sacrificed for harvest of *M. leprae* during DDS treatment, the mice were first exsanguinated and plasma was separated and stored frozen for later analysis. DDS analyses were carried out by the chromatographic-fluorometric method (Murray *et al.,* 1971, 1975).

Results

The results of 2 typical experiments, portrayed in Fig. 1, demonstrate the application of the 2 criteria to characterize the antimicrobial activity of DDS when the drug administered in a 90-day "pulse" beginning during logarithmic

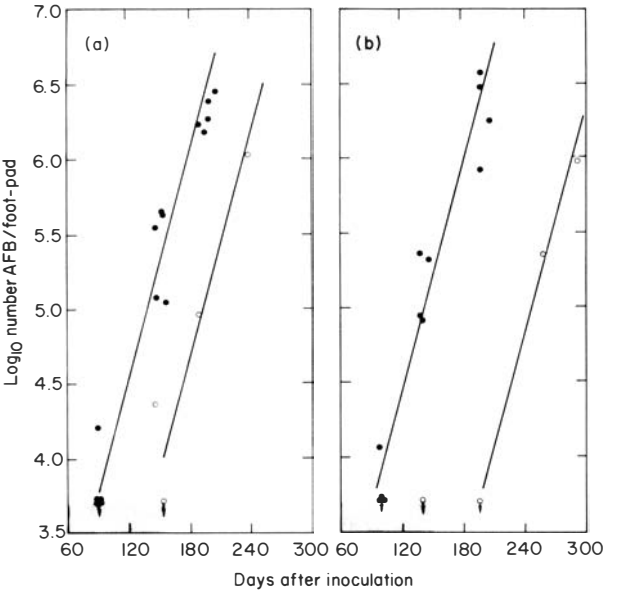

Fig. 1. Log_{10} number of AFB (*M. leprae*) per foot-pad as a function of the time after inoculation of mice. Mice were inoculated with $10^{3.7}$ organisms in both hind foot-pads on day 0. Those of experiment m6-12-68 (a) were administered 10^{-4} g% DDS incorporated into the mouse chow; the mice of experiment m4-2-69 (b) were administered 10^{-2} g% DDS. The drug was administered for the time indicated by the shaded bars along the abscissae. In each experiment: (●) results of harvests of *M. leprae* from pools of the tissues of 4 foot-pads of untreated control mice; (○) results of harvests of *M. leprae* from the foot-pads of treated mice. The solid lines, the best-fitting straight lines drawn through the collections of points with a slope equivalent to a doubling time of 12 days, represent the logarithmic phase of bacterial multiplication. No organisms were found in the harvests represented by the points with the downward-extending arrows; the points were calculated as if one organism had been encountered in the counting procedure.

multiplication of *M. leprae*. In experiment m6-12-68 (experiment no. 1 of Table 1), 10^{-4} g% DDS was administered for 95 days, beginning 61 days after the mice were inoculated. The regression line representing logarithmic multiplication of the organisms in untreated mice yields values of 178 days for the time after inoculation at which multiplication achieved the level of 10^6 *M. leprae* per foot-pad, and $10^{3.02}$ for the number of organisms per foot-pad at the time DDS administration was begun. Harvests of *M. leprae* from the foot-pads of treated mice during the period of DDS administration 146 and 153 days after inoculation yielded $10^{4.38}$ and fewer than $10^{3.70}$ AFB per foot-pad respectively. The geometric mean of these 2 values, $10^{4.04}$, represents the level to which *M. leprae* had multiplied in treated mice after DDS administration was begun. This level was reached in untreated mice 102 days after inoculation and 41 days after treatment had been started. Thus, there was a delay of 41 days between beginning treatment and the onset of inhibition. The number of doublings of *M. leprae* during this delay may be determined:

$$10^{4.04}/10^{3.02} = 10.5\text{-fold multiplication; } \log_2 10.5 = 3.39 \text{ doublings.}$$

The regression line representing logarithmic multiplication of *M. leprae* in treated mice after withdrawal of DDS yields a value of 233 days for the time at which multiplication reached the level of 10^6 AFB per foot-pad. Multiplication of the organisms in untreated mice had reached this level 55 days earlier. DDS had been administered for 95 days, but the *M. leprae* had continued to multiply during the first 41 days of administration, leaving a period of 54 days during which DDS inhibited multiplication of the organisms. Thus, the *M. leprae* appear to have resumed multiplication virtually immediately upon cessation of DDS administration. Therefore, in this experiment, in which mice were treated with 10^{-4} g% DDS beginning during logarithmic multiplication of the *M. leprae,* the organisms continued to multiply through about 3.4 doublings before the onset of inhibition, and multiplication resumed immediately when DDS treatment was stopped.

In experiment m4-2-69 (experiment no. 4 in Table 1), 10^{-2} g% DDS was administered for 88 days beginning 61 days after inoculation. At this time, the calculated number of AFB per foot-pad was $10^{2.97}$. A harvest of *M. leprae* from the foot-pads of treated mice on day 139 yielded fewer than $10^{3.70}$ AFB per foot-pad. Because multiplication of *M. leprae* in untreated mice appears to have reached the level of $10^{3.70}$ AFB per foot-pad 90 days after inoculation and 29 days after beginning DDS administration, multiplication of *M. leprae* in the treated mice could have continued for no longer than 29 days after treatment was begun. During this time, no more than 2.4 doublings of *M. leprae* could have occurred, assuming a doubling time of 12 days. Once DDS treatment had been stopped, multiplication to the level of 10^6 AFB per foot-pad appears to have occurred 105 days later in treated than in untreated mice. At least 46 days—105-(88-29)—represents a delay of resumption of multiplication that cannot be accounted for by the presence in the mouse tissues of DDS in an effective concentration. Thus, in this experiment in which mice were treated with 10^{-2} g% DDS, multiplication of *M. leprae* probably ceased immediately upon the institution of treatment, and resumed only after a considerable delay, once treatment was stopped.

The results of 20 experiments in which DDS was administered to *M. leprae*-infected mice are presented in chronological order in Table 1. The data in this table were derived by means of the calculations just described. As shown by the second column of the table, the drug was administered in a dosage of 10^{-4} g% in 18 experiments, and in other dosages in 5 experiments. For each of the 20 experiments, the number of AFB per foot-pad at the time DDS administration was started is shown in column 3. In the fourth column are entered the actual results of harvests of *M. leprae* from the foot-pads of treated mice about 90 days after beginning treatment; DDS was stopped at this time in all but one experiment. The fifth column of Table 1 shows the number of days elapsed between inoculation and multiplication of *M. leprae* to the level of 10^6 per foot-pad in untreated mice. The number of days between the start of treatment and multiplication of *M. leprae* to the level of 10^6 AFB per foot-pad in untreated mice, which is the difference between the values in column 5 and the number of days elapsed between inoculation and the start of DDS administration, is shown in the sixth column. The purpose of the data of columns 5 and 6 is to permit the consideration of the point on the growth curve of *M. leprae* at which the administration of DDS was begun. The concentration of DDS measured in the plasma of mice sacrificed for harvest of *M. leprae* during treatment is shown in the

TABLE 1

Characteristics of the effects of treatment with DDS on the multiplication of M. leprae *in the mouse foot-pad*

Experiment no.	Dosage of DDS* (10^{-4} g%)	Log_{10} AFB/foot-pad		Time to 10^6 AFB/foot-pad From inoculation† (days)	From start of DDS† (days)	Plasma DDS (μg/ml)	No. of doub-lings	Delay § (days)
		Start of DDS†	End of DDS‡					
1	1.0	3.01	<4.03	178	117	<1.0	<3.4	1
2	1.0	3.82	4.85	148	86	–‖	3.4	17
2	10	3.82	3.76	148	86	–‖	0	31
2	100	3.82	<3.70	148	86	–‖	0	68
3	100	2.96	4.33	180	120	–‖	4.6	103
4	100	2.97	<3.70	181	120	–‖	<2.4	46
5	1.0	3.73	5.39	150	89	–‖	5.5	>156
6	0.3	3.97	5.36	155	80	<1.0	4.6	8
6	1.0	3.97	5.48	155	80	2.0	4.9	39
7	1.0	3.55	4.61	171	96	1.8	3.5	–¶
8	1.0	4.40	5.51	139	63	4.0	3.7	60
9	1.0	5.06	5.87	114	38	1.5	2.7	>328
10	1.0	4.94	5.96	103	42	6.4	3.4	<1
11	1.0	4.21	5.09	129	70	5.2	2.9	106
12	1.0	4.43	5.30	120	61	6.9	2.9	72
13	1.0	4.73	5.66	110	50	6.2	3.0	191
14	1.0	4.43	4.88	115	53	4.2	1.5	92
15	1.0	4.43	5.42	121	61	3.1	3.2	53
16	1.0	4.33	4.76	128	66	9.2	1.4	55
17	1.0	3.94	4.27	143	81	6.5	1.0	22
18	1.0	4.46	5.33	123	62	6.8	2.9	76
19	1.0	4.12	4.73	134	74	8.4	2.0	27
20	1.0	4.24	4.70	131	69	6.2	1.5	37

* DDS was administered incorporated in the mouse chow for about 90 days, beginning about 60 days after inoculation in all experiments except nos 6-9, in which drug administration was begun about 75 days after inoculation.

† Calculated from the regression line representing the logarithmic phase of bacterial multiplication in untreated control mice.

‡ Actual number recovered at harvest from treated mice.

§ Delay = the difference between the time to 10^6 AFB per foot-pad in treated mice and that in untreated mice (column 5), from which has been subtracted the difference between the duration of treatment and the duration of continued multiplication after beginning DDS.

‖ Not measured.

¶ Not applicable, because treatment was not stopped after 90 days.

seventh column. In the eighth column of Table 1 is presented the number of doublings of *M. leprae* that occurred in treated mice after treatment had been started. In the last column is shown the duration of the delay of resumption of multiplication in treated mice after cessation of treatment that cannot be attributed to the presence of DDS in the tissues in effective concentration.

Multiplication of *M. leprae* appears to have ceased immediately after beginning treatment with 10^{-2} g% DDS in experiment no. 2 and probably also in experiment no. 4. In experiment no. 3, the harvest of *M. leprae* performed just before withdrawal of treatment yielded $10^{4.34}$ AFB per foot-pad, representing 24-fold multiplication or 4.6 doublings. However, 2 subsequent harvests performed 194 and 233 days after inoculation yielded fewer than $10^{3.70}$ and $10^{3.70}$ AFB per foot-pad respectively. These data suggest that the yield of the first harvest was not representative, and that multiplication of *M. leprae* had indeed ceased immediately after treatment with 10^{-2} g% DDS had been begun in this experiment also. In each of these 3 experiments, once treatment had been withdrawn, multiplication of *M. leprae* resumed only after a delay.

In the one experiment in which mice were treated with 10^{-3} g% DDS (experiment no. 2), multiplication appears to have ceased immediately after treatment had been started, and resumption of multiplication of *M. leprae* appears to have been delayed significantly after cessation of treatment. In contrast, in the one experiment in which mice were treated with $10^{-4.5}$ g% DDS (experiment no. 6), *M. leprae* continued to multiply through almost 5 doublings after treatment had been begun, and multiplication resumed without significant delay after DDS administration was stopped. Thus, the results of treatment of mice with DDS in dosages of $10^{-4.5}$, 10^{-3} 10^{-2} g% appear consistent with those reported by Shepard (1967, 1969): the larger the dosage, the fewer the doublings after beginning treatment and the longer the delay of resumption of multiplication after withdrawal of treatment.

The major problem to which this study is addressed is the lack of uniformity of the results of treatment with 10^{-4} g% DDS. Multiplication of *M. leprae* was inhibited in all 18 experiments. The number of doublings of *M. leprae* after beginning DDS administration varied fron 1.0-5.5, with a mean of 2.93 doublings. The relationship of the number of doublings to several characteristics of bacterial multiplication is shown in Table 2. It is apparent that the time required from inoculation to multiplication to 10^6 AFB per foot-pad in untreated mice varied from experiment to experiment, probably as a result of variation of the proportion of viable organisms in the inoculum or of the duration of the lag phase. Because treatment was started at about the same time after inoculation in all experiments, the portion of the logarithmic phase during which DDS administration was started also differed considerably from experiment to experiment. Neither the time from inoculation to multiplication to 10^6 AFB per foot-pad nor the time from beginning treatment to multiplication to this level was correlated with the number of doublings of *M. leprae* after beginning treatment. The number of doublings was negatively correlated with the concentration of DDS measured in the mouse plasma, and also with the date on which the experiments were started.

The duration of the delay of resumption of multiplication of *M. leprae* also varied from experiment to experiment in which 10^{-4} g% DDS was administered. There was no delay in experiments no. 1 and 10; the duration of the delay was barely significant in experiments no. 2, 17 and 19; and the delay was longer than

TABLE 2

Correlation of the number of doublings and the delay of resumption of multiplication with several characteristics of multiplication of Myco. leprae

Correlation examined	*r**	*t**	*P**
Number of doublings versus:			
Log_{10} AFB foot-pad:			
At start of DDS	−0.244	1.01	> 0.05
At end of DDS	0.435	1.93	> 0.05
Time to 10^6 AFB foot-pad:			
From inoculation	0.352	1.51	> 0.05
From start of DDS	0.271	1.12	> 0.05
Plasma DDS concentration†	−0.587	2.71	< 0.02
Date experiment initiated‡	−0.760	4.68	< 0.01
Number of days delay versus:			
Log_{10} AFB foot-pad:			
At start of DDS§	0.490	2.18	< 0.05
At end of DDS‖	0.521	2.36	< 0.04
Time to 10^6 AFB/foot-pad:			
From inoculation	−0.379	1.59	> 0.05
From start of DDS¶	−0.482	2.13	0.05
Plasma DDS concentration	−0.269	1.01	> 0.05
Date experiment initiated	−0.033	0.13	> 0.05
Number of doublings	0.126	0.49	> 0.05

* *r*, The correlation coefficient; *t*, Student's "*t*"; *P*, probability.

† Number of doublings = 2.74 − (0.24 ± 0.19) (DDS concentration in ng/ml − 4.96). In this and the subsequent regression equators, the expression for the slope includes the 95% confidence limits around the estimate of the slope of the regression line.

‡ Number of doublings = 2.93 − (0.36 ± 0.16) (date experiment initiated − 7.3).

§ Number of days delay = 78.4 + (83.8 ± 81.8) (log_{10} AFB per foot-pad at start of DDS − 4.24).

‖ Number of days delay = 78.4 + (79.8 ± 72.2) (log_{10} AFB per foot-pad at end of DDS − 5.12).

¶ Number of days delay = 78.4 + (2.084 ± 2.081) (number of days from start of DDS to 10^6 AFB per foot-pad − 68.4).

100 days in experiments no. 5, 9, 11 and 13. The duration of the delay may be seen in the lower panel of Table 2 to have been correlated with the numbers of AFB per foot-pad at the beginning of DDS administration and at the end of the period of treatment in treated animals, and to have been negatively correlated with the time elapsed from start of treatment to multiplication to the level of 10^6 AFB per foot-pad in untreated mice. None of these correlations is striking, however. The duration of the delay was not correlated with plasma DDS concentration, the date experiments were initiated, or the number of doublings that occurred after beginning treatment.

Discussion

The purposes of this study were to describe the manner in which multiplication of *M. leprae* in the mouse foot-pad was affected by a 90-day course of DDS begun during the logarithmic phase of bacterial growth, and to find an explanation for

the variation of the effects of treatment from experiment to experiment. Our results appear to confirm those reported earlier by Shepard (1967, 1969). Administration of DDS in a concentration of 10^{-4} g% inhibited multiplication of this strain of *M. leprae.* The bacterial strain used in this study proved also to be susceptible to $10^{-4.5}$ g% DDS; in an earlier, unpublished study, it was found to multiply in mice during the administration of 10^{-5} g% DDS. These results are consistent with the earlier demonstration that *M. leprae* recovered from untreated patients are uniformly susceptible to DDS in this dosage, and that many strains of *M. leprae* recovered from untreated patients are susceptible to $10^{-4.5}$ g% DDS, whereas only a few are susceptible to 10^{-5} g% DDS (Shepard *et al.,* 1969; Levy and Peters, 1976).

Our results also demonstrate the relationship described earlier (Shepard, 1967, 1969) between the dosage of DDS on the one hand and characteristics of drug action on the other—the lag between beginning treatment and the onset of inhibition of multiplication, and the delay of resumption of multiplication of *M. leprae* after the cessation of DDS administration. These 2 phenomena are of particular interest because of the variable effects noted to follow the administration of DDS in a concentration of 10^{-4} g%.

Multiplication has been reported to continue through several generations after the addition of sulphonamides to cultures of susceptible organisms (Wolff and Julius, 1939). The authors speculated that the lag between addition of drug and onset of growth inhibition depended upon the presence within the organisms of a store of an essential substance, the supply of which is affected by the drug. The concentration-dependence of this phenomenon was not described in this report, however. Our demonstration that the duration of the lag in the case of inhibition of *M. leprae* by DDS is related to the concentration of DDS appears more consistent with the presence within the organisms of a store of a precursor of di- or tetrahydrofolic acid than with a store of the end-product itself.

The results of administration of 10^{-4} g% DDS demonstrate considerable variation of the 2 characteristics of DDS action from experiment to experiment. The number of doublings of *M. leprae* that occurred after the start of DDS administration was inversely related to the concentration of DDS in the plasma of mice sacrificed near the end of the period of treatment, and also to the date on which the experiments were started. That the number of doublings was smaller when a larger DDS concentration was measured is not unexpected. That the number of doublings was smaller in more recent experiments requires explanation. In another study, conducted during a portion of the time covered by this study, variation of the plasma DDS concentration from experiment to experiment was noted, although no trend could be discerned (Levy and Peters, 1976). However, in the present study, the plasma DDS concentration was positively correlated with the date on which the experiments were begun (r, the correlation coefficient = 0.744; Student's $t = 4.17$; $P < 0.0003$). Thus, the relationship between the number of doublings of *M. leprae* after beginning treatment and the plasma DDS concentration at the time mice were sacrificed for harvest is consistent with the relationship between the number of doublings and the date on which mice were inoculated in each experiment. The date on which the experiments were started is also correlated negatively with the time from inoculation to multiplication to the level of 10^6 AFB per foot-pad in untreated mice ($r = 0.615$; $t = 3.12$; $P < 0.002$). Although this relationship may suggest that our technique has drifted during the course of the past 8 years, no relationship could be discerned between the number of doublings that occurred in treated

mice after treatment had been started and the time from inoculation to multiplication to 10^6 AFB per foot-pad of untreated mice.

The duration of the delay of resumption of multiplication of *M. leprae* after administration of 10^{-4} g% DDS was stopped also varied among experiments. The duration of the delay was positively correlated with the number of AFB per foot-pad at the beginning of treatment and negatively correlated with the time from beginning of treatment to multiplication to the level of 10^6 *M. leprae* per foot-pad of untreated mice. These findings are consistent, because both measurements are made from the same regression line. On the other hand, the duration of the delay was found to be correlated with the number of AFB per foot-pad of treated mice measured near the end of the period of treatment, whereas it was not correlated with the number of doublings between the start of treatment and the onset of growth inhibition. These findings are inconsistent, because the number of doublings is determined by the difference between the number of AFB per foot-pad of treated mice mice at the end of the period of treatment and the number of AFB per foot-pad at the time DDS administration was begun. The duration of the delay was correlated neither with plasma DDS concentration (in the case of those experiments in which 10^{-4} g% DDS was administered) nor with the date experiments were begun.

Taken together, these results are consistent with the explanation that both characteristics of DDS effect—the lag between beginning treatment and onset of inhibition, and the delay of resumption of multiplication after the end of treatment—depend upon the dosage of DDS. However, the number of doublings of *M. leprae* after the start of DDS treatment appears to be more sensitive to relatively minor variations of the concentration of DDS in the neighbourhood of 10^{-4} g% than is the duration of the delay of resumption of multiplication once treatment has been stopped.

References

Goldstein, A. (1964). *Biostatistics.* New York: Macmillan.

Levy, L. (1976). Studies of the mouse foot-pad technique for cultivation of *Mycobacterium leprae.* 3. Doubling time during logarithmic multiplication. *Lepr. Rev.* **47**, 103.

Levy, L. and Peters, J. H. (1976). Susceptibility of *Mycobacterium leprae* to dapsone as a determinant of patient response to acedapsone. *Antimicrob. Ag. Chemother.* **9**, 102.

Murray, J. F., Jr, Gordon, G. R. and Peters, J. H. (1971). A chromatographic-fluorometric procedure for the determination of nanogram quantities of antileprotic sulfones. *J. Lab. clin. Med.* **78**, 464.

Murray, J. F., Jr, Gordon, G. R., Gulledge, C. C. and Peters, J. H. (1975). Chromatographic-fluorometric analysis of antileprotic sulfones. *J. Chromat.* **107**, 67.

Shepard, C. C. (1960). The experimental disease that follows the injection of human leprosy bacilli into foot-pads of mice. *J. exp. Med.* **112**, 445.

Shepard, C. C. (1967). A kinetic method for the study of activity of drugs against *Mycobacterium leprae* in mice. *Int. J. Lepr.* **35**, 429.

Shepard, C. C. (1969). Further experience with the kinetic method for the study of drugs against *Mycobacterium leprae* in mice. *Int. J. Lepr.* **37**, 389.

Shepard, C. C. and McRae, D. H. (1968). A method for counting acid-fast bacteria. *Int. J. Lepr.* **36**, 78.

Shepard, C. C., Levy, L. and Fasal, P. (1969). The sensitivity to dapsone (DDS) of *Mycobacterium leprae* from patients with and without previous treatment. *Am. J. trop. Med. Hyg.* **18**, 258.

Shepard, C. C. and McRae, D. H. (1971). Hereditary characteristic that varies among isolates of *Mycobacterium leprae. Infect. Immun.* **3**, 121.

Wolff, L. K. and Julius, H. W. (1939). Action du sulfanilamide *in vitro* et *in vivo. Annals Inst. Pasteur, Paris* **62**, 616.